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BY

NELLIE A. BROWN

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THE TENDENCY OF THE CROWN-GALL ORGANISM TO PRODUCE ROOTS IN CONJUNCTION WITH TUMORS[1]

By NELLIE A. BROWN

Associate Pathologist, Office of Horticultural Crops and Diseases, Bureau of Plant Industry, United States Department of Agriculture

INTRODUCTION

In his early work with the crown-gall organism, *Bacterium tumefaciens*, Erwin F. Smith made definite reference to the production of roots by the apple strain of that organism (*20*).[2] Later, when writing about the analogy of plant to animal cancer through the formation of teratomata (*17*, *18*), he described and pictured many leafy shoots on or along the sides of tumors produced by inoculating tobacco, Bryophyllum, and geranium with several different strains of the organism. He attributed these abnormal leafy shoots, flower buds, and root formations to the kind of tissues inoculated, together with the growth conditions of the plant at the time of inoculation, and he thought that the diffusible products of the bacteria within a growing tumor could stimulate surrounding uninoculated tissues to multiply. His interest in showing the analogy of plant to animal tumors influenced him to work along the more striking line of shoot production.

The stimulation to root production by the various strains of *Bacterium tumefaciens* has been somewhat overlooked. This statement does not refer to the apple strain, however, which has been recognized since 1908 as a strain that produces a mass of roots (fig. 1) when it enters a wounded or poorly grafted apple root. Some investigators maintain that there are two types of apple strain, one that produces only tumors and another that produces only masses of roots. In the writer's opinion, which is based on years of observation and investigation, there is only one apple strain, and in this paper, which deals largely with the apple strain, the crown gall and infectious hairy root of apple are considered as the same disease, which manifests itself as a tumor, a mass of roots, or a combination of both. It happens that in those cases where roots occur in clumps without a definite tumor, inconspicuous swellings are usually present.

REVIEW OF LITERATURE

In his work with crown gall, which embraced hundreds of experiments in orchards and nurseries, Hedgcock (*3*) in 1905 established the pathological effect of crown gall on orchard trees. He produced many cross infections with minced galls from peach, apricot, almond, apple, chestnut, and other trees by inserting pieces in wounds on seedling trees planted in sterile soil, and found that by this method he could produce infection readily on some hosts. The apple, however, was infected with difficulty, and when galls of the apple were transferred to other fruit trees infection occurred only on a limited

[1] Received for publication Apr. 1, 1929; issued November, 1929.

[2] Reference is made by number (italic) to "Literature cited," p. 765.

FIGURE 1.—Hairy root on Windsor apple from Nebraska. Photographed November, 1927. One-half natural size

number of hosts (*4*). Hedgcock noted a marked difference in the susceptibility of different varieties of apple trees to crown gall and he found that infection on some varieties tended to the gall type and on others to the hairy-root type. He did not establish the cause of these outgrowths on fruit trees. He reported that not all the malformations on apple trees were due to an infectious agent, but that many were caused by poor grafting unions which resulted in an excess of callus formation (*5*).

In 1907 the bacterial origin of the crown gall on the Paris daisy was established by Smith and Townsend (*19*), and the causal organism was named *Bacterium tumefaciens*. In 1911 it was further determined that the organism had the ability to cause tumor growths on plants in many unrelated families. One of these hosts was the apple, on which the disease was manifested by a definite gall or a small outgrowth from which a mass of roots projected (*20*).

The malformations on apple stems reported years ago in England are considered by horticulturists there and by Charles F. Swingle in the United States as varietal characteristics. They have been designated as burr knots (*2, 8, 21*).

The writer made many attempts to isolate *Bacterium tumefaciens* from burr knots on apple but without success (*1*). Undoubtedly there are outgrowths on apple stems caused by *Bact. tumefaciens*, but the writer believes that the burr knot or stem tumor of apple is produced by some other agent.

Studies of outgrowths on grafted apple trees and proof of the production of callus formation without the intervention of *Bacterium tumefaciens*, and other related crown-gall investigations, have been carried on by Riker, Keitt, Melhus, and Muncie, working alone or in groups (*6, 7, 9, 10, 11, 12, 13, 14*). They induced overgrowths on grafted apple trees at the scion tips, using aseptical methods with the stock and scion cuttings to reduce as far as practicable or completely eliminate the presence of any organism. In their isolation studies with apple galls they obtained what appeared to be *Bact. tumefaciens* colonies on poured plates, but except in a very few cases inoculations with subcultures failed to produce the outgrowths necessary for proof. The percentages ran low even when isolations were made from 100 to 200 and more apple galls. The plants used for testing the pathogenicity of these isolations were mostly tomato, but tobacco and geranium were also used. These three are all hosts on which it is difficult to produce tumors with the apple strain, although tumors are produced readily on them with some other crown-gall strains. In fact, there is no quick way of testing the pathogenicity of *Bact. tumefaciens* isolated from apple crown gall. The Paris daisy, *Impatiens balsamina*, and string-bean stems have been found by the writer, as noted elsewhere in this paper, to become infected with the apple strain in less time than is required to produce outgrowths on the apple stems, but usually three to seven weeks must be allowed even for these hosts.

Recently Siegler (*16*) also isolated *Bacterium tumefaciens* from galls on apple trees and proved its pathogenicity by producing roots and outgrowths on apple and various other hosts. His isolations also showed the slowness and inadequacy of the apple strain in producing definite roots and sizable tumors on tobacco, geranium, and tomato plants.

As shown in some of the publications cited, the malformations on apple trees are classified under several names, the one called noninfectious hairy root being considered by some workers the most prevalent type.

Riker, Banfield, Wright, and Keitt (*15*) recently isolated from apple crown gall an organism which produced roots when inoculated into apple trees but which failed to produce infection on tomato plants. They inoculated 15 strains isolated from 15 different sources into young apple shoots, and all produced roots; the organism was reisolated from the enlargements at the bases of these roots. This the writer considers the typical apple strain of crown gall, which will not infect tomato, or at least will not produce a conspicuous infection, until it has passed through and been isolated from another host. What Riker and his associate call a root-stimulating organism, as though it were unrelated to crown gall, is the apple strain, which in reality is a root-stimulating organism. The apple strain showed this tendency in 1908 when the writer first isolated it from both galls and hairy roots on apple trees. It possesses this strain difference just as it also possesses similarities to other strains of crown gall, and the writer does not think it should be placed under any new classification.

THE APPLE STRAIN OF BACTERIUM TUMEFACIENS

ROOT-PRODUCING ABILITY

The general tendency of the crown-gall organism isolated from an apple tree naturally infected with hairy root is to produce roots when inoculated into an apple tree (figs. 2, A*a*, and 3, B), but it can produce definite tumors (fig. 2, A*b*) and some flat outgrowths covered or partially covered with root primordia (fig. 2, D).

This ability of the apple strain to stimulate the production of roots is not confined to infections on the apple tree, for it readily stimulated root production on stems of the Paris daisy (fig. 4, A*a*, A*b*), Bryophyllum (fig. 5, B*a*), and sugar-beet root (fig. 6, A). Not every successful inoculation produces roots, and a tumor may form without roots, as is shown on the Bryophyllum. (Fig. 5, B*b*.) Sugar beets inoculated with the apple strain produce roots as the usual type of infection, but frequently there are tumors of various sizes present. Figure 6, B, shows a definite tumor produced on a sugar beet inoculated with the hop strain and, growing in the same bed, a sugar beet inoculated at the same time with the apple strain. (Fig. 6, A.)

Tumors without roots were produced on rose and bean plants by the same culture that produced roots on daisies and sugar beets. Inconspicuous root primordia were present on the tumors, but the surfaces were not covered by them.

An organism isolated from a natural rootless tumor of apple produced roots and definite sizable tumors on Paris daisy stems. (Fig. 5, D and C.) It also produced definite tumors on apple trees. (Fig. 3, C.) An organism isolated from an induced tumor (without roots) on an apple tree can produce both tumors and roots. This was proved by inoculating sugar beets with such an isolation. Masses of roots occurred at the inoculation places on eight of them, and in addition tumors occurred on four.

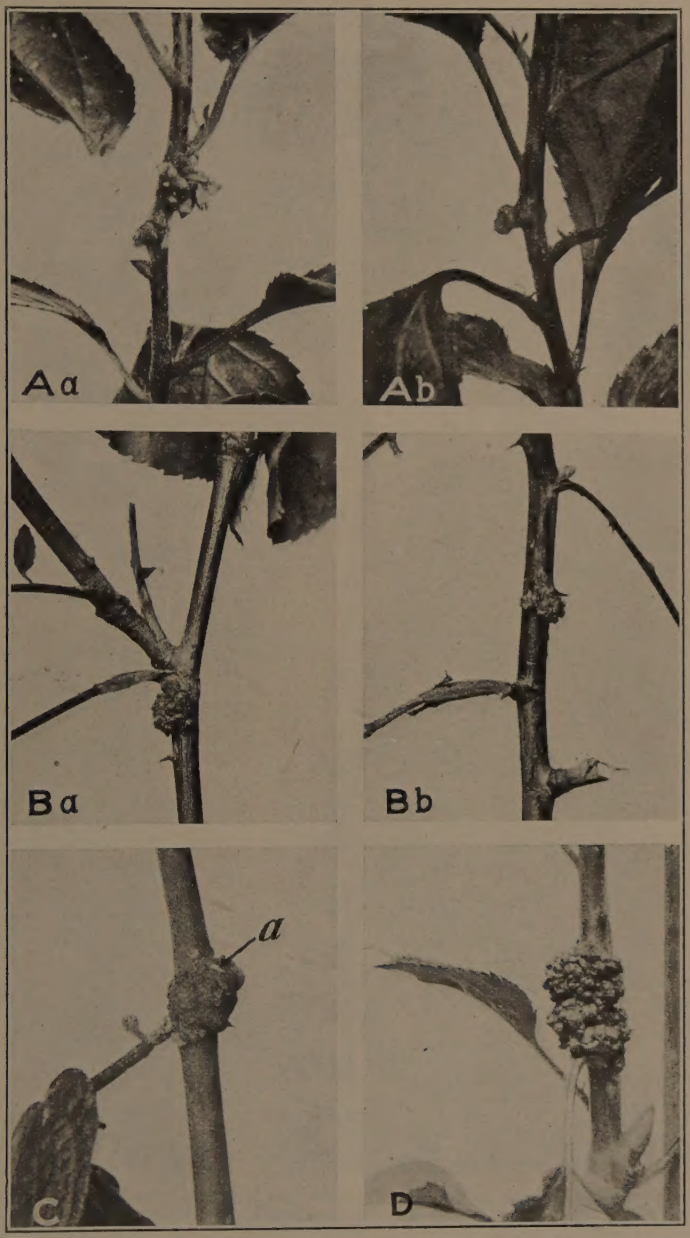

FIGURE 2.—Inoculations of various hosts with the organism isolated from an apple hairy root. All natural size: A, Seedling apple trees, two months after inoculation; A*a*, tumor with roots; A*b*, tumor without roots. B*a* and B*b*, Rose stems, five months after inoculation. C, Stem of string bean, three months after inoculation. Small white root, *a*, developed after plant had been kept in refrigerator at 10° to 14° C. for 20 hours. D, Seedling apple tree, seven months after inoculation

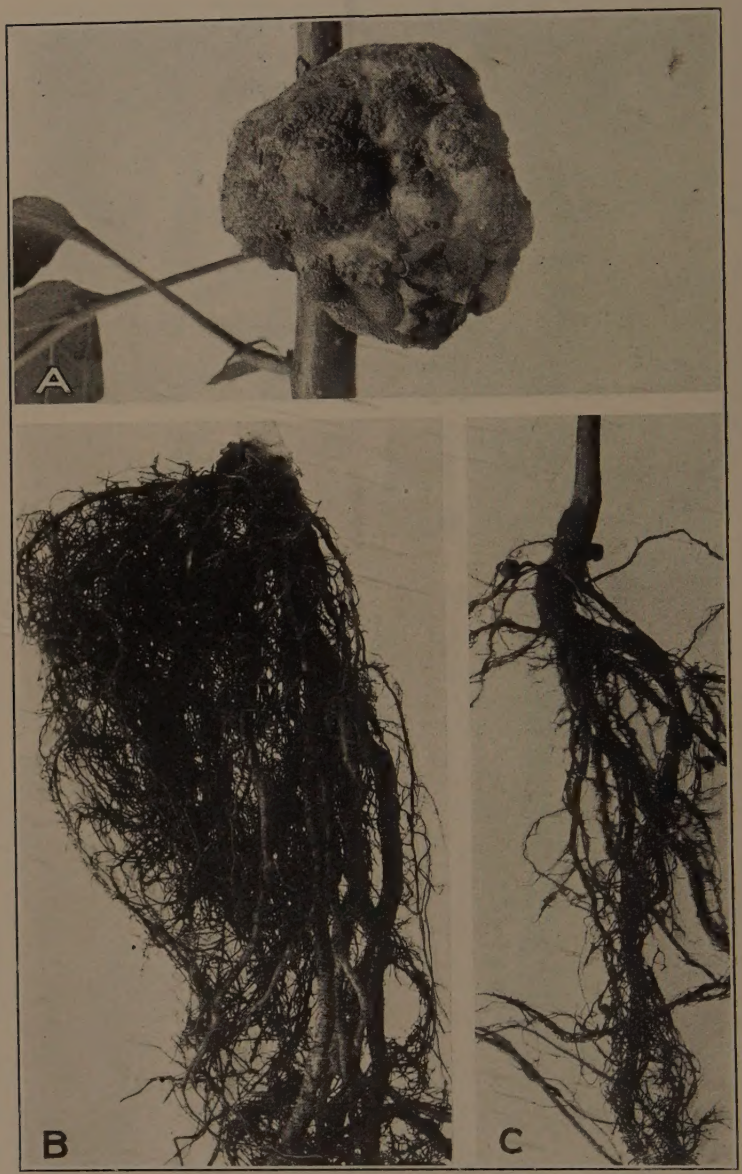

FIGURE 3.—A, The peach strain of *Bacterium tumefaciens* inoculated into a growing stem of an apple tree. Time, five months. Three-fourths natural size. B, Apple tree inoculated at crown with organism isolated from the hairy-root type of infection, showing hard tumor and masses of roots. Time, nearly five months. Reduced in size. C, Apple tree inoculated at crown with organism isolated from tumor on apple without the root masses, and in this case producing only tumors. Time, two months. Reduced in size. The same isolation produced masses of root primordia on Paris daisy stems (fig. 5, D) and a definite tumor with roots on Paris daisy at crown (fig. 5, C)

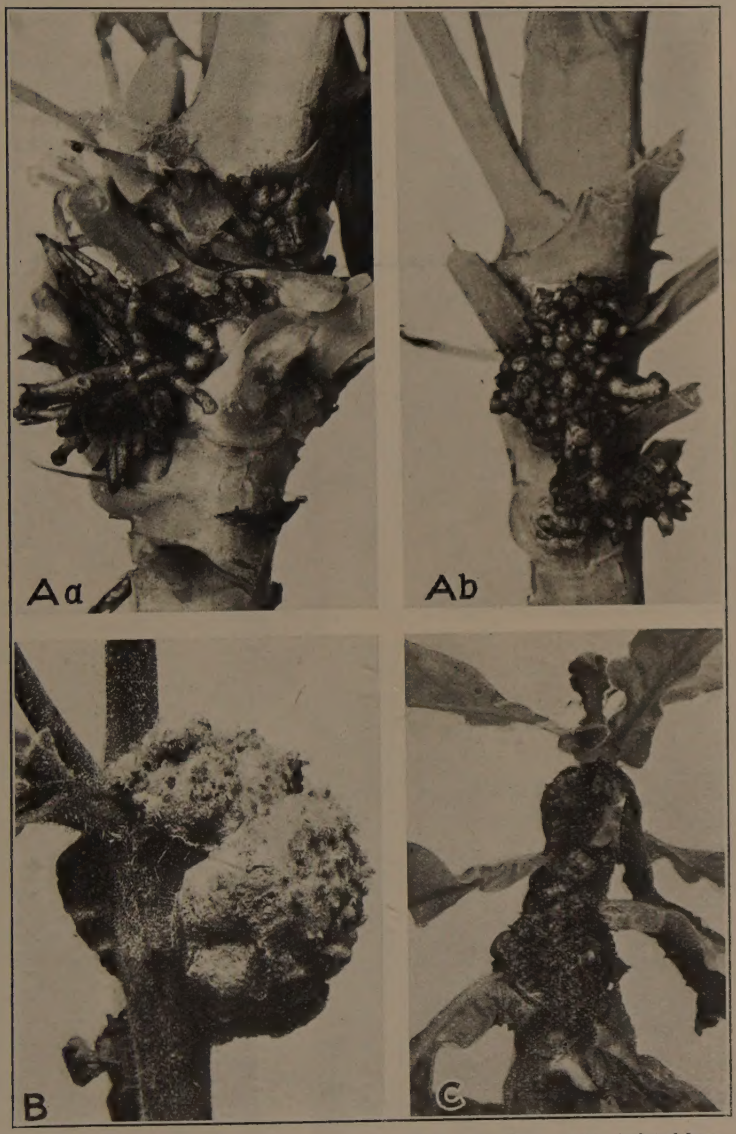

FIGURE 4.—A*a*, and A*b*, Paris daisy stems inoculated with the organism isolated from hairy root of Windsor apple shown in Figure 1, November 23, 1927. Photographed March 3, 1928. × nearly 2. B, Tobacco stem inoculated with rose strain of *Bacterium tumefaciens* showing tumor with root primordia. Time, two months. × nearly 2. C, Tobacco stem inoculated with peach strain of *Bact. tumefaciens* showing tumor with roots. Time two months. Natural size

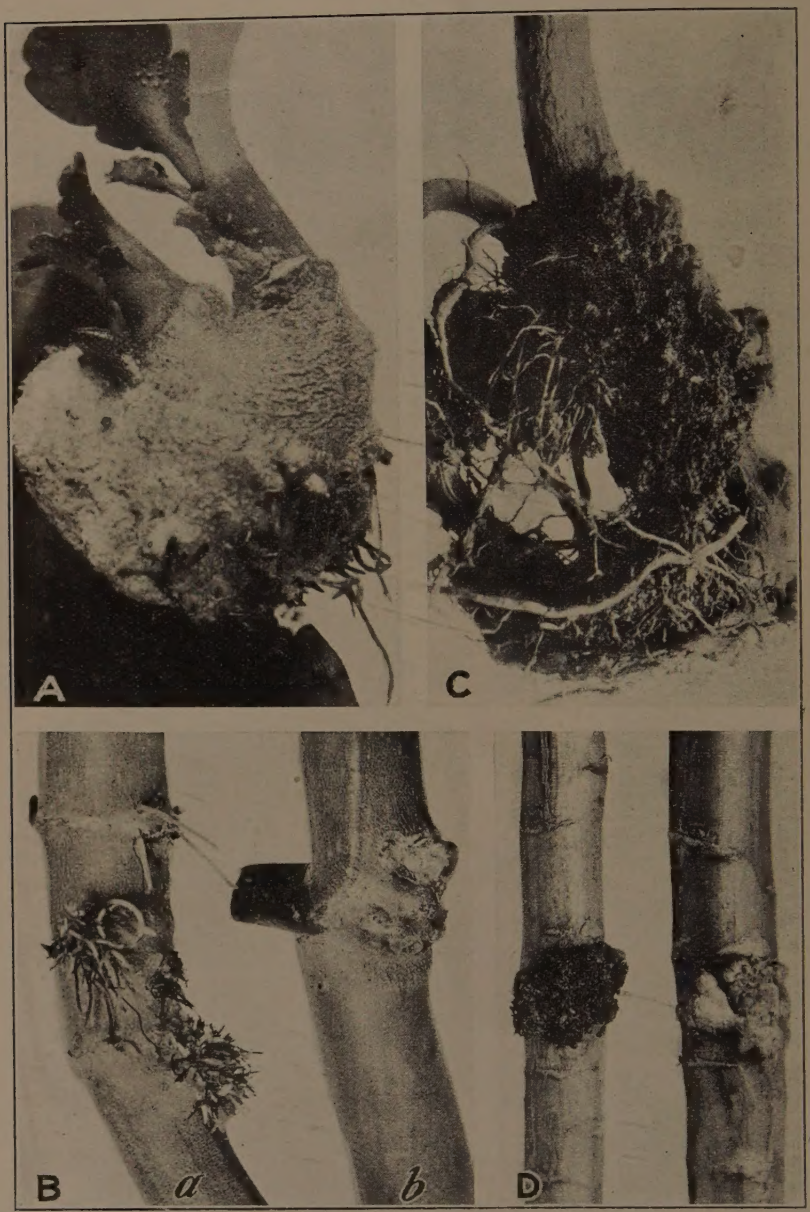

FIGURE 5.—A, *Bryophyllum pinnatum* inoculated with the hop strain of *Bacterium tumefaciens*, showing tumor and roots. Time, three months. × nearly 5. B, *Bryophyllum pinnatum* inoculated with the apple hairy-root strain of *Bact. tumefaciens*. The stem with the tumor and no definite roots, B*b*, was inoculated with the original isolation colony. The stem with roots, B*a*, was inoculated with a reisolation colony on the same date. Time of both, five months. Natural size. C, Tumor with roots on Paris daisy produced by inoculating the crown with an organism isolated from a definite tumor on apple (in distinction from hairy root). Time, 10 months. Natural size. Figure 3, C, shows the same isolation inoculated into apple crown. D, Tumor and masses of root primordia produced by inoculating stems of Paris daisy with the same organism used to produce C. Time, eight months. Natural size

FIGURE 6.—A, Sugar beet inoculated with the apple hairy-root strain of *Bacterium tumefaciens*, March 27, 1928. Photographed June 23, 1928. Tumors and roots produced. B, Sugar beet inoculated with the hop strain at the same time and photographed on the same date as A. Only a tumor produced. Natural size

THE APPLE TREE AS A HOST

The apple tree is a less favorable host to the crown-gall organism than the peach and apricot, on whose tender cortex tumors form more readily. But it is not entirely due to the less tender tissues of apple that tumors or hairy roots form more slowly on it than on peach and apricot. The appearance of tumor growths on rapidly growing apple seedlings inoculated with the apple strain usually requires two months, which is twice as long, or even longer, than the time required for tumors to appear on peach or apricot trees inoculated with the peach and apricot strains of *Bacterium tumefaciens*. Even the active peach strain when inoculated into apple trees is a slow tumor producer, but it is much more rapid than the apple strain. (Fig. 3, A.) The slowness of the peach strain in producing tumors on apple trees may be due to the more acid condition of the apple stem, as indicated by the pH, 4.6. The peach stem has a pH value of 4.8. The tests of both peach and apple stems were made near the close of the summer, however, and there might have been a wider difference in the pH values had the tests been made at some other time of the year.

The slowness of the apple strain in producing tumors on its native host is characteristic of its action on the other hosts to which it is infectious. When young, quickly growing daisy plants are inoculated with the apple strain by using subcultures soon after isolating, the tumors or roots do not appear even as swellings until six or seven weeks have elapsed, and growth continues slowly. Figure 4, A*a* and A*b* show daisy stems three and one-half months after inoculation with this strain. The peach, daisy, apricot, gooseberry, pecan, honeysuckle, and some other strains show infections on the daisy within a week and sometimes less after inoculation. The hop strain will not produce a tumor on the daisy, although the daisy strain will produce a tumor rather readily on the hop root or stem.

The apple strain isolated from the hairy root illustrated in Figure 1 did not produce so many infections on its natural host as did the peach, hop, and daisy strains on theirs. These last three strains may produce 90 to 100 per cent infection. A weakly pathogenic colony of the apple strain may produce less than 20 per cent infection on apple trees, and an infectious colony 40 to 50 per cent. The same colony may cause 60 to 70 per cent infection (or even more) on Paris daisy and *Impatiens balsamina*. Reisolation colonies from apple-crown gall on daisy produce as high as 80 per cent infection. Ten infectious reisolation colonies obtained from roots on daisy (fig. 4, A*a*), when inoculated into 54 daisy stems, produced roots on 9 (fig. 7, A, B, D); tumors on 34, some with root primordia (fig. 7, C); and no infection on the remaining 11.

REACTIONS OF VARIOUS HOSTS TO INOCULATIONS

Young string-bean plants inoculated with the apple hairy-root isolation did not soon produce outgrowths, as four weeks or longer elapsed before definite swellings appeared. Bean plants of the same age inoculated at the same time with the hop strain developed 6-mm. tumors within three weeks. When bean stems were inoculated with the apple hairy-root isolation definite tumors rather than roots or a combination of roots and tumors were produced.

FIGURE 7.—A–D, Paris daisy stems inoculated, March 13, 1928, with four different colonies reisolated from outgrowths shown in Figure 4, A*a*. Photographed May 12, 1928. All natural size

When the rose was inoculated with the cultures used for beans and daisies it was slow to show infection, and when very tender shoots were inoculated infection did not appear for six weeks or longer. The type of outgrowth was a tumor with root primordia.

The castor-bean plant (*Ricinus communis*) was thought for some time to be immune to the apple strain. One set of inoculations appeared to be negative after two months. While another set of inoculations was in progress to check up this earlier one, small outgrowths appeared on the first set three months after inoculation, and on the second set between two and three months after inoculation. The tumors never grew larger than 4 mm. across, but they were definite infections which might easily have been overlooked if the controls had not been so definitely without swellings. The percentage of infection was about 50. Some of the Ricinus stalks were split longitudinally through the inoculated area, and little masses of tumors were found in the pith extending into the hollow center at these points. To make absolutely certain that the external and internal outgrowths were caused by the organism that had been inserted, platings were made from the tiny outside tumors and from the pith outgrowths, and crown-gall colonies appeared on both sets of plates. Subcultures made from colonies on these two sets of plates produced tumors when inoculated into daisies.

Ricinus is an acid plant with a pH of 5.4, but the peach and apple stems are more acid (pH 4.8 and 4.6, respectively), and the apple strain causes infection on them. Evidently there is some other feature of Ricinus than its hydrogen-ion concentration, perhaps a high titratable acidity, which makes it unfavorable as a host. The hop and peach strains, however, found Ricinus a very favorable host and produced tumors three-fourths to 1 inch in diameter in four weeks on vigorous plants.

Geranium and tobacco, like Ricinus, are favorable hosts to many strains of *Bacterium tumefaciens*, but are only slightly susceptible to the apple strain. For some time these plants were considered immune to the apple strain. Small flat inconspicuous outgrowths (4 to 5 mm. in diameter) of the tumor type were produced only under the most favorable conditions in two to four months, and but a small percentage of the inoculations caused infection (3 out of 24 with geranium and 4 out of 24 with tobacco). No root growths or tumors with roots were produced. Figure 8, D, shows the results of inoculating tobacco with the isolation from the hairy root shown in Figure 1.

DISTINCTIVE FEATURES

Isolations of the apple strain produce fewer virulent colonies, and the strain has a more limited number of host plants than any of the other strains except the rose. Slowness in producing tumors seems to be inherent in the strain itself, for slowness is characteristic of its action on its own host, the apple, as well as on other hosts. Moreover, the organism is slow in its growth in culture media. Like the rose strain, it is less translucent on beef and Thaxter (potato-dextrose) agar than other strains, which makes it appear in some stages quite unlike the crown-gall organism. In no medium used by the writer does it produce such a heavy growth as the other strains of *Bacterium tumefaciens*. Most isolations of the rose strain must be excepted, for this strain likewise failed to produce a heavy growth in the media used.

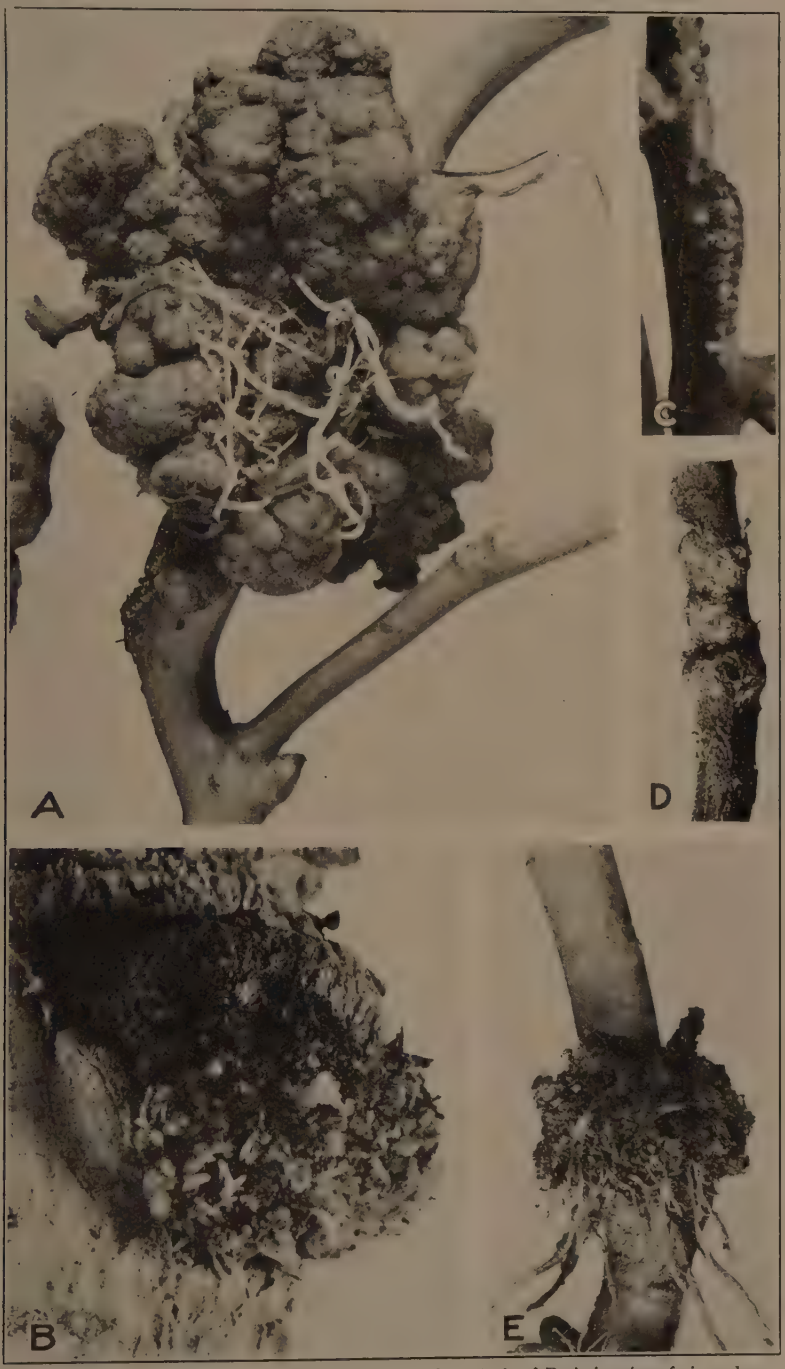

FIGURE 8.—A, Nasturtium stem inoculated with the hop strain of *Bacterium tumefaciens*, showing tumor and roots. Time, three months. Nearly natural size. B, Collards stem inoculated with poplar strain, showing tumor and roots. Time, two months. × 2. C, Tomato stem inoculated with daisy strain, showing tumors with a few root primordia. Time, five weeks. Natural size. D, Tobacco stem inoculated with the apple strain isolated from the apple hairy root shown in Figure 1. Time, five months. Natural size. E, Geranium inoculated with chrysanthemum strain, showing tumor and roots. Time, one year. Natural size

The apple and rose strains are alike (1) in producing comparatively few virulent isolation colonies, (2) in their habit of growth and appearance in culture media, (3) in having a more limited number of host plants than the other strains, and (4) in their slowness to produce tumors, although some tumors eventually become as large as the average tumor produced by other strains.

That the crown-gall organism is able to adapt itself to new conditions was shown by the apple strain in its reisolation colonies. The reisolations were made from apple hairy root produced on daisies. (Fig. 4, A*a*.) When these reisolation colonies were inoculated into other daisy stems infection appeared sooner than in the original inoculations. While with the original organism it took nearly two months for the inoculations to show infection, with the reisolation colonies it took a few days less than one month. The tendency to produce roots as well as definite tumors was retained in the reisolation colonies. (Fig. 7, A–D.) Of the 10 colonies picked off and tested, all proved infectious to daisy stems, and 80 per cent of the 54 inoculations were positive.

The writer has never been able to produce a definite swelling on tomato plants with the apple strain, although she has made inoculations in top and base and in the neighborhood of adventitious roots and has used plants of various ages and isolations and reisolations from different sources. Thirty-six tomato plants inoculated with the isolation from the hairy root shown in Figure 1 gave negative results. Small inconspicuous swellings with smooth surfaces were produced on tomato stems inoculated with a reisolation from apple tumor on daisy. The tomato stem is a favorable host to the peach and hop strains, but only moderately so to the daisy strain. (Fig. 8, C.) Tobacco is a favorable host to the rose (fig. 4, B), peach (fig. 4, C), and hop strains. If the peach strain of *Bacterium tumefaciens* in the soil should pass through the apple crown or root and produce infection, an isolation from this apple-peach tumor would probably still be able to produce an infection on the tomato stem as the original organism does. In his work with teratomata Smith (*18*) used the hop strain largely, and he pictured many hop tumors on tobacco.

The cultural characteristics of most of the reisolation colonies changed. The reisolations grew more rapidly and more abundantly in culture media than the original isolations, and on beef and potato-dextrose agar they looked more like the vigorous peach and hop strains than like the original isolations.

Further proof of the adaptability of the crown-gall organism was shown by the reisolation of the apple strain from tumors on Ricinus. The inconspicuous outgrowths which took three months to form on Ricinus produced reisolation colonies which developed outgrowths on daisy stems more rapidly than the apple-strain reisolations made from the daisy itself. The Ricinus reisolations of apple strain when used for inoculations showed good infections in the daisy in two to three weeks, whereas it took the daisy reisolations (apple strain) almost four weeks. Furthermore, the reisolation of the apple strain from Ricinus, like the reisolation of the apple strain from daisy, produced colonies which grew much more vigorously in culture media than the original apple isolation colonies, to which they were related.

It might be mentioned here that the strain isolated from a natural tumor on the crown of a hop plant became much more virulent and made a heavier growth in culture media after it was isolated from a hop tumor induced on a sunflower plant.

ROOT PRODUCTION WITH OTHER STRAINS OF BACTERIUM TUMEFACIENS

Although the apple alone of all the crown-gall strains seems to possess the inherent ability to produce roots whether it enters the crown, root, or stem of the apple tree or some other susceptible plant, still there are other strains of *Bacterium tumefaciens* that will produce roots on certain plants and under certain conditions. These roots are usually in combination with tumors and are produced mostly on plants that can be propagated easily from cuttings.

The hop strain produces roots very readily on *Impatiens balsamina*, the common garden balsam. The roots grow on or near the tumor and may be from 5 to 25 mm. or more in length. (Fig. 9, B.) The peach and poplar strains also produce roots on *I. balsamina* in less time than it takes the apple strain to do so. (Figs. 9, A, and 10, A–C.) It is very likely that all the strains of *Bacterium tumefaciens* will produce roots on this susceptible host and that each will display some individuality in its root production, as is shown by the four strains pictured.

One month after the balsam stems were inoculated with the hop strain, roots 10 to 12 mm. long had been produced, and the tumors were 1 to 1.5 cm. in diameter, or even larger. The roots on the stems of the plants inoculated with the poplar strain were not so long, and those inoculated with the peach strain were much shorter, being only 3 to 4 mm. In one month the inoculations, made with the original isolation from the apple hairy root and with two reisolations, showed tiny tumor swellings 3 to 4 mm. across with root primordia but no root extensions. The photographs showing root extensions of apple strain were made two months after inoculation. The experiment was repeated, but the apple strain was less active and only a few roots extended from the tumors even after three months. The three other strains showed vigorous roots.

The masses of roots often produced at the crown of chrysanthemum plants and the abnormal number of roots on rooted cuttings of chrysanthemum have been found to be produced in some cases by *Bacterium tumefaciens*. These masses of roots are not beneficial to the plant, for the vigor and substances that should go into the production of leaves and flowers are used in the thickened masses of roots. The organism isolated from chrysanthemum roots produces both roots and tumors when inoculated into susceptible plants. An example of a tumor with roots on a geranium produced by inoculating it with the chrysanthemum strain is shown in Figure 8, E.

The hop strain inoculated into Bryophyllum and nasturtium stems can produce definite tumors with roots. (Figs. 5, A, and 8, A.) So also can the poplar strain inoculated into collards stems. (Figs 8, B.) Occasionally the daisy strain will produce a few roots from the tumors on daisy stems.

FIGURE 9.—*Impatiens balsamina* inoculated June 15, 1928: A, With peach strain of *Bacterium tumefaciens;* B, with hop strain. Photographed September 17, 1928. Both natural size

FIGURE 10.—*Impatiens balsamina* inoculated with different strains of *Bacterium tumefaciens*, June 15, 1928, and photographed September 17, 1928: A, Poplar strain; B, apple hairy-root strain; C, reisolated strain from apple hairy root. All natural size

STIMULATION OF ROOT PRIMORDIA TO ROOT FORMATION

Neither the rose nor the bean stems inoculated with the isolation from the apple hairy root produced roots in conjunction with the tumors (figs. 2, B*a*, B*b*, and C), but some of the tumor surfaces were roughened somewhat by root primordia. The bean tumors were stimulated to root production by chilling the whole plant in the refrigerator at a temperature of 10° to 14° C. for 20 hours, and then exposing it to a temperature of 25° to 27°. The primordia would extend into little roots 2 to 3 mm. long in four to eight hours. Some of the primordia that did not develop into roots in 24 hours could be made to do so by several sucessive changes from cold to warm temperatures. The change from cold to warm temperatures seemed to be necessary, as a pot of bean plants left in the refrigerator for 5 successive days did not show any root extensions from the three tumors until it had been in a warm room for about six hours. The tumors with smooth surfaces did not respond to the treatment, for roots did not appear.

The rose tumors with root primordia were stimulated to produce roots by being covered with sphagnum moss which was kept moist. Three of the eight tumors covered had produced roots when the sphagnum was removed after seven days. The stimulus here may have been a change of temperature also, for in a hot greenhouse evaporation must take place from the moist sphagnum; this could reduce the temperature of the tumor and might cause a stimulation to root development.

The roots induced by the ice-box and moss-ball stimulation were from 2 to 8 mm. in length. In some cases they were longer, but the tips dried back in the warm room as the root extended and true measurements could not be made, The roots of the rose tumors may have formed in a short time, but a week had elapsed when the moss ball was removed. One root from a rose tumor was 15 mm. long when uncovered, but the root was too fragile to survive the period required for photographing.

DISCUSSION

Can this tendency to root production of the crown-gall organism be an indication of a primitive soil form and the first manifestation of its pathogenicity? And, further, can it be that after passing through the apple or some other host and back into the soil the organism shows more active pathogenic features? And if so, why could not these more active features be manifested in the more definite tumor form instead of roots? This first act in pathogenicity might explain to some extent the slow adaptation of *Bacterium tumefaciens* to the apple as a host and the more frequent hairy-root type of crown gall which has been so confusing to nurserymen, and it might also explain the real tumor form of crown gall on apple to be produced by an organism beyond the first adaptation stage to the apple as host.

The organism reisolated from apple hairy root on daisy (fig. 4, A*a*) produces roots and tumors more rapidly than the original isolation, and it grows more rapidly and profusely in culture media than does its forebear. That the crown-gall organism in general is an adaptive one is shown by the many plants that it infects, for with the exception of the monocotyledons, outgrowths caused by it have been found on most of our common fruits, vegetables, and ornamental plants.

The fasciation of stems of various plants like the sweet pea and the nasturtium known to be caused by the crown-gall organism might be produced by the same soil pathogenic form demonstrating its pathogenicity at the crown of a plant by fasciation instead of root or tumor production. The writer found that the organism isolated from fasciated sweet-pea stems was a weakly pathogenic one which would infect tiny sweet-pea seedlings but not plants 5 or more inches tall. The percentage of infection in the seedlings was only 37.

It is not known why some isolations of the apple strain are weakly parasitic and others actively parasitic. It may be that the less active organism is the primitive crown-gall parasite, or that it has had to survive a long residence in the soil between the times of host residence, while the more active one has passed through and infected one or more apple trees in succession and so increased its virulence. Or it may be that those very active crown-gall strains such as peach, hop, and daisy have had still more passages through various hosts, each time becoming more adaptable and virulent before returning to the soil.

SUMMARY

The crown-gall organism (*Bacterium tumefaciens*) can stimulate plants to produce roots as well as tumors. The strain isolated from apple trees produces both roots and tumors. Both the hairy-root and the tumor types of infection produced by the apple strain are considered symptoms or manifestations of the same disease.

The apple strain is slower to infect susceptible hosts than other strains except the rose. It is also slow to infect the apple tree.

The Paris daisy and *Impatiens balsamina* respond to inoculations with the apple strain by producing masses of roots and small tumors; the rose and bean stems by producing definite tumors instead of masses of roots; *Bryophyllum pinnatum* by producing masses of roots and tumors without roots. Tobacco, geranium, and Ricinus are very indifferent hosts on which a few inconspicuous outgrowths without roots are produced in two to four months. The results on tomato were negative.

When first isolated the apple strain differs somewhat in appearance from the other crown-gall strains (the rose excepted) when grown in culture media. These differences disappear when the apple strain is passed through another host and reisolated. These reisolation colonies tend to produce infection in less time than the orginal colonies.

Like the other strains of *Bacterium tumefaciens*, the apple strain is an adaptable one (though less so than the other strains), and when it passes through another host than the apple it becomes more virulent.

Root production is not limited to the apple strain of *Bacterium tumefaciens*, for the peach, hop, poplar, chrysanthemum, and other strains also produce roots.

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